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Contribution to the knowledge of the beetle fauna (Insecta, Coleoptera) of Malta: new records of seven species with supporting DNA barcodes

Clare Marie Mifsud[1], Noel Vella[1], Adriana Vella[1*]

1 Conservation Biology Research Group, Biology Department, University of Malta, Msida, MSD 2080, Malta • CMM: cmifsud2@gmail.com https://orcid.org/0000-0003-4605-7400 • NV: noel.vella@um.edu.mt https://orcid.org/0000-0003-0080-5709 • AV: adriana.vella@um.edu.mt https://orcid.org/0000-0003-2246-5354

* Corresponding author

Abstract

We report the presence of seven species of Coleoptera newly found on the Maltese Islands in the central Mediterranean. The first species records from the Maltese Islands include: *Ancylopus melanocephalus* (Olivier, 1808) (Endomychidae), *Aplidia transversa* (Fabricius, 1801) (Scarabaeidae), *Cercyon quisquilius* (Linnaeus, 1761) (Hydrophilidae), *Hyperaspis duvergeri* Fürsch, 1985 (Coccinellidae), *Lebia cruxminor* (Linnaeus, 1758) (Carabidae), *Smicronyx pauperculus* Wollaston, 1864 (Curculionidae), and *Oxytelus sculptus* Gravenhorst, 1806 (Staphylinidae). The morphological identification of each newly reported species was also confirmed through DNA barcoding.

Keywords

Carabidae, Coccinellidae, Curculionidae, Endomychidae, Hydrophilidae, Scarabaeidae, Staphylinidae

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Introduction

Coleoptera is the most diverse animal order on Earth. More than 386,000 extant beetle species have been described and represent around 25% of all described animal species (Bouchard et al. 2017). Due to this immense diversity, listing the actual number of described species from all world regions remains challenging, and estimating the number of undescribed species is even more so.

Cameron and Caruana Gatto (1907) comprehensively listed around 600 Coleoptera species from the Maltese Islands, and 100 years later the total number of species increased to 800 (Mifsud 2000a). An estimate of 1800 species of Coleoptera has been indicated for the Maltese Islands (Mifsud 2000a), and although an updated catalogue is still unavailable, progress has been made with updated species lists for several coleopteran families (reviewed by Schembri 2003). The groups to which we add species newly recorded from the Maltese Islands have been reviewed and updated in previous works and include the families Endomychidae, Scarabaeidae, Hydrophilidae, Coccinellidae, Carabidae, Curculionidea, and Staphylinidae (Bordoni 1973; Schembri and Schembri 1982; Schembri 1993; Magrini and Schembri 1997; Mifsud and Vigna Taglianti 1999; Shockley et al. 2009; Mifsud and Colonnelli 2010; 2018; Pivotti et al. 2011; Przewoźny 2018).

Complete species lists from restricted areas such as islands are useful by providing information on biodiversity at a finer scale to better guide conservation efforts. Therefore, this research aims to extend our knowledge on the coleopteran fauna of the Maltese Islands to support biodiversity conservation efforts.

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Methods

A beetle collection was compiled by the Conservation Biology Research Group, University of Malta (CBRG-UM) between 2015 and 2019. All beetles were either hand collected during the day or captured at night in UV light traps set in the field from May to October of each sampling year. Beetle specimens were collected from various habitat types including urban and rural areas across the islands of Malta and Gozo (Fig. 1). Beetle specimens were individually stored in labelled sampling bottles and placed at –20 °C on the same day of collection until further processing. Each specimen was examined and photographed. Morphologically identified species were checked for their occurrence in Malta using the Fauna Europaea database portal (de Jong et al. 2014), published catalogues, and literature (Mifsud and Scupola 1998; Mifsud 2000b, 2002; Mifsud and Knizek 2009; Mifsud and Colonnelli 2010; Pivotti et al. 2011; Lillig et al. 2012; Mifsud and Jelínek 2012; Nardi and Mifsud 2015; Sabella and Mifsud 2016; Sogoh and Yoshitomi 2017; Haran 2018; Mifsud and Nardi 2020). Only beetles not previously recorded from the Maltese Islands are included in this work.

For DNA barcoding of voucher specimens, genomic DNA was extracted using the whole body of small beetle specimens (<3 mm body length). Three legs and one leg were used for DNA extraction from medium-sized beetle specimens (3–10 mm body length) and large specimens (>10 mm body length), respectively. Genomic DNA extraction was done using the GF-1 Tissue DNA Extraction Kit (Vivantis, Malaysia) following the manufacturer's manual. The 658 bp DNA barcode region of the mitochondrial cytochrome *c* oxidase subunit I gene (COI) was amplified using a cocktail mix of the Folmer primers LCO1490/HCO2198 (Folmer et al. 1994) and LepF1/LepR1 (Hebert et al. 2004) appended with the universal M13 oligonucleotide tails. Amplification reactions were done in 25 µL reaction volumes, containing 1 µL genomic DNA (approximately 10 ng), 0.25 µL of each primer pair (0.5 µM, each), 1 µL of 25 mM MgCl2, 5 µL 1X FIREPOL Master Mix containing 2.5 mM Mg^{2+}, 200 µM of each dNTP and 1 U FIREPOL DNA polymerase (Solis BioDyne, Estonia) and 17.5 µL ddH2O. PCR reactions were carried out on a Nexus Gradient Mastercycler (Effendorf, Hamburg, Germany) using the following temperature profile: 95 °C for 5 min; followed by 6 cycles of 95 °C for 45 s, 45 °C for 30 s, 72 °C for 1 min and 36 cycles of 95 °C for 45 s, 50 °C for 30 s, 72 °C for 1 min and final extension at 72 °C for 15 min. 2 µL of each PCR product and 100 bp DNA ladder (Solis BioDyne, Estonia) were visualised on a 1.5% agarose gel stained with ethidium bromide to confirm amplification and concentration. Good quality PCR products were subsequently purified and sequenced with the respective PCR forward and reverse primers using an ABI3730XL sequencer.

Quality check, editing, and alignments of the resulting DNA sequences were done in Geneious v. 11.1.2 (https://www.geneious.com; Kearse et al. 2012). DNA barcode sequences were aligned using MUSCLE (Edgar 2004), primer nucleotide sequences were removed, and chromatograms were checked for the presence of double peaks, stop codons or frameshifts which could indicate the amplification of nuclear mitochondrial (NUMT) pseudogenes. Molecular species identification was

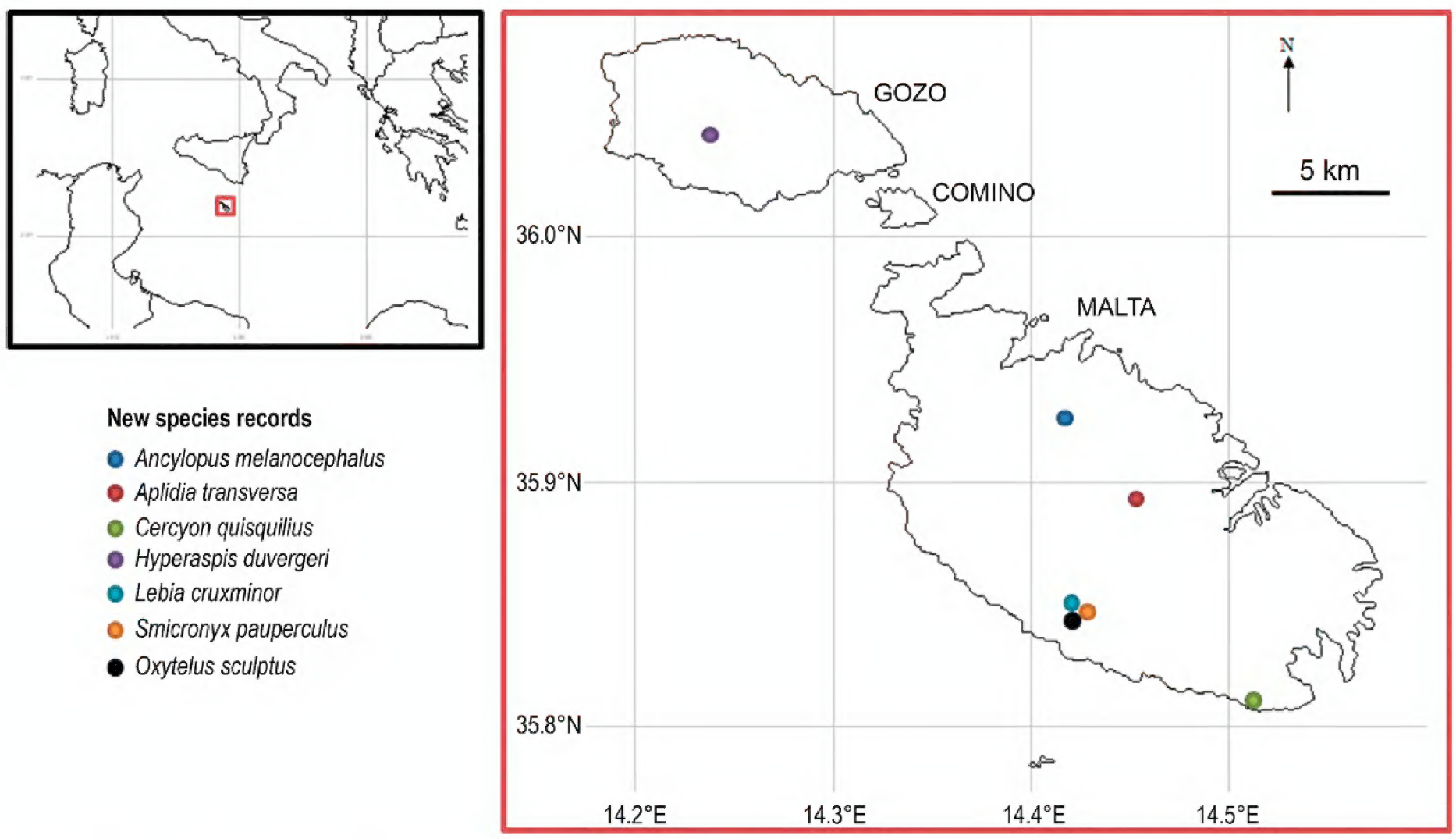

Figure 1. Location of the Maltese Islands within the Mediterranean Sea and a distribution map of the newly recorded seven beetle species across the islands of Malta and Gozo.

carried out by comparing the newly generated DNA barcodes to those available in public databases. The public databases used include the Nucleotide collection (nr/nt) of the NCBI GenBank database (https://www.ncbi.nlm.nih.gov/genbank; Benson et al. 2012) and the species level barcode records available at the Barcode of Life Data System (BOLD, http://www.boldsystems.org; Ratnasingham and Hebert 2007). Comparison of the query DNA sequences to the databases was done using BLASTn v. 2.9.0 (Zhang et al. 2000; Morgulis et al. 2008) in GenBank and using the identification portal system in BOLD. A species delimitation genetic distance threshold value of 2% was used.

Results

Family Endomychidae

Ancylopus melanocephalus (Olivier, 1808)

Figure 2A

New records. MALTA – **Malta** • Żebbiegħ; 35°55′14.17″N, 014°22′58.38″E; 33 m a.s.l.; 1.VIII.2018, C. M. Mifsud leg.; UV light trap; Mediterranean stream dominated by *Arundo donax*; 2 ♂, CBRG-UM T10T11 • same locality; 8.IX.2018; 6 ♂, CBRG-UM T60; 4 ♀, CBRG-UM T59; GenBank: MT908984; 1 ♀, CBRG-UM BTS26.

Identification. Body elongate-oval, 5.0–5.5 mm long; elytra with three black maculae laterally and a longitudinal band along the sutural line; elytral basal edge thickened and raised. Female pronotum with lateral sulci connected medially by an arcuate ridge. Male mesofemur with a fringe of long hairs along inner edge. *Ancylopus melanocephalus* (Olivier, 1808) can be distinguished from *Ancylopus ceylonicus* Strohecker, 1971, the most similar species, by the mesotibia of males, which is slightly widened and without a tooth in *A. melanocephalus* (El Torkey and Al Dhafer 2015). The elytra are also a distinguishing feature between the two similar species. The elytra have three black maculae laterally and a longitudinal band along the sutural line in *A. melanocephalus* while in *A. ceylonicus* the elytral suture, apex, and side margin are black with a large mediolateral elytral spot in males (El Torkey and Al Dhafer 2015). Molecular species identification analyses resulted in the highest pairwise identity of 96.8% with *Ancylopus pictus asiaticus* Strohecker, 1972 (KU188382) from GenBank, while no species level barcode records were available from BOLD. The molecular species identification results confirm the genus of our specimen and represent the first DNA barcode for the species *A. melanocephalus*.

Distribution. Saudi Arabia (El Torkey and Al Dhafer 2015); Bulgaria, Hungary, Spain, Italy (Shockley et al. 2009), and Malta (newly recorded).

Family Scarabaeidae

Aplidia transversa (Fabricius, 1801)

Figure 2E

New record. MALTA – **Malta** • Attard; 35°53′27.34″N, 014°26′24.07″E; 90 m a.s.l.; 9.VI.2017; N. Vella leg.; urban habitat; GenBank: MH510725; 1 ♀, CBRG-UM Col77.

Identification. *Aplidia transversa* (Fabricius, 1801) can be distinguished from the other five species within the genus following the work of Baraud (1992). Body length 15.5 mm, dark red-brown coloration, base of the pronotum without long erect hairs, pronotum punctured in a fine, regular, and simple pattern, hair on elytra very short, shorter than the intervals between the punctures. Molecular species identification analyses resulted in the highest pairwise identity of 99.6% with *Aplidia transversa* (KM449893) from GenBank and also matched the BIN (Barcode Index Number) for *Aplidia transversa* [BOLD:ACD1022] from BOLD. The molecular species identification further confirms the morphological identification of the examined specimen.

Distribution. Albania, Austria, Bosnia and Herzegovina, Bulgaria, Croatia, Germany, Greece, France, Italy, Macedonia, Montenegro, Romania, Serbia, Slovenia, Switzerland, Turkey (Baraud 1992), and Malta (newly recorded).

Family Hydrophilidae

Cercyon quisquilius (Linnaeus, 1761)

New record. MALTA – **Malta** • Ħal Far; 35°49′07.75″N, 014°30′42.26″E; 46 m a.s.l.; 20.VII.2018; C. M. Mifsud leg.; UV light trap; Mediterranean cliff and open habitat; GenBank: MT908996; 1♂, CBRG-UM BTS101.

Identification. Body length 2.5 mm; dorsal surface of head black; elytra brownish yellow without dark sutural spot. Mesoventral plate narrower, 6× as along as wide. Metaventrite without femoral lines and with raised pentagonal area markedly wide at midlength. *Cercyon quisquilius* (Linnaeus, 1761) can be confused with *Cercyon nigriceps* (Marsham, 1802) but can be distinguished from it in the coloration of the pronotum, which is blackish with diffuse yellowish areas on lateral margins in *C. quiquilius*, while in *C. nigriceps* it is reddish-brown, similar to the elytral coloration. *Cercyon quiquilius* is also larger than *C. nigriceps* (2.4–3.2 mm vs 1.0–2.1 mm; Arriaga-Varela et al. 2017). Our molecular species identification analyses using the DNA barcode sequence resulted in a pairwise identity of 99.8% with *C. quisquilius* (KU911068) from records on GenBank and also matched the BIN for *C. quisquilius* [BOLD:AAH0271] from BOLD, further confirming our morphological species identification.

Distribution. Native to the Palaearctic region, including Malta (newly recorded). Introduced to Australia, Mexico, Argentina, Hawaii, the Caribbean (Cuba), and China (Arriaga-Varela et al. 2017).

Figure 2. Dorsal habitus. **A.** *Ancylopus melanocephalus* (Olivier, 1808). **B.** *Smicronyx pauperculus* Wollaston, 1864. **C.** *Oxytelus sculptus* Gravenhorst, 1806. **D.** *Lebia cruxminor* (Linnaeus, 1758). **E.** *Aplidia transversa* (Fabricius, 1801).

Family Coccinellidae

***Hyperaspis duvergeri* Fürsch, 1985**

New record. MALTA – **Gozo** • Fontana; 36°02′11.15″N, 014°14′06.17″E; 70 m a.s.l.; 21.V.2019; N. Vella leg.; Mediterranean stream dominated by *Arundo donax*; GenBank: MT909025; 1 sex indet., CBRG-UM P5_39.

Identification. Body uniformly oval, 3.2 mm long; pronotum black in the middle with orange lateral margins; elytra black with a single pair of orange spots in the posterior half of the elytra. These morphological characteristics separate *H. duvergeri* from other species within the genus *Hyperaspis* using the key by Biranvand et al. (2017). Our molecular species identification analyses resulted in the highest pairwise identity of 91.0% with *Hyperaspis postica* LeConte, 1880 (MF633070), from GenBank, while no species level barcode records were matched on BOLD. Our DNA barcode represents the first DNA barcode record for *H. duvergeri*.

Distribution. Iran (Moddarres-Awal 1997); Algeria, Morocco, Portugal, Spain, France, Italy, Croatia, Hungary (Kovář 2007), and Malta (newly recorded).

Family Carabidae

***Lebia cruxminor* (Linnaeus, 1758)**

Figure 2D

New record. MALTA – **Malta** • Fawwara; 35°50′19.75″N, 014°24′40.06″E; 185 m a.s.l.; 12.VII.2018; C. M. Mifsud leg.; UV light trap; Mediterranean garrigue habitat; GenBank: MT909027; 1 sex indet., CBRG-UM BTS144.

Identification. Body length 6.0 mm; elytra with dark, transverse band and presence of a scutellar spot, apical margin of elytra dark; legs partially blackened. Our molecular species identification analyses resulted in the highest pairwise identity of 99.1% with *Lebia cruxminor* (Linnaeus, 1758) (KU911743) from GenBank. The molecular species identification on BOLD matched our sequence to the BIN [BOLD:ACB0195] assigned to *L. cruxminor*, further confirming our morphological species identification through DNA barcoding.

Distribution. Europe (de Jong et al. 2014), including Malta (newly recorded), Iran (Azadbakhsh and Nozari 2015), Russia (Egorov et al. 2020), and Japan (GBIF Secretariat 2021a).

Family Curculionidea

***Smicronyx pauperculus* Wollaston, 1864**
Figure 2B

New records. MALTA – **Malta** • Fawwara; 35°50′19.75″ N, 014°24′40.06″E; 185 m a.s.l.; 31.VII.2018; C. M. Mifsud leg.; UV light trap; Mediterranean garrigue habitat; GenBank: MT909077; 1 sex indet., CBRG-UM BTS173 • same locality; GenBank: MH510770; 1 sex indet., CBRG-UM Col250.

Identification. Body length 2.0 mm. Body integument reddish except rostrum, antennae, prothorax, suture of elytra, and tarsi, which are black; vestiture of elytra consisting of elongate, brownish scales not concealing integument and white scales, thicker, forming transverse patches; rostrum moderately downcurved in lateral view (Haran 2018). Our molecular species identification analyses resulted in the highest pairwise identity of 98.6% with *Smicronyx pauperculus* Wollaston, 1864 (KU942313) from GenBank and matched the BIN for *S. pauperculus* [BOLD:ACS7419] from BOLD, thereby supporting the morphological species identification through DNA barcoding.

Distribution. Mediterranean basin and Africa (Haran et al. 2017; Haran 2018), including Malta (newly recorded).

Family Staphylinidae

***Oxytelus sculptus* Gravenhorst, 1806**
Figure 2C

New record. MALTA – **Malta** • Fawwara; 35°50′19.75″ N, 014°24′40.06″E; 185 m a.s.l.; 12.VII.2018; C. M. Mifsud leg.; UV light trap; Mediterranean garrigue habitat; GenBank: MT909046; 1 sex indet., CBRG-UM, voucher ID BTS150.

Identification. Body length 3.0 mm; antennomere 4 notably baso-dished; antennomere 1 stout; supra-antennal ridges elevated (Lü and Zhou 2015). Our molecular species identification analyses resulted in the highest pairwise identity of 99.6% with *Oxytelus sculptus* Gravenhorst, 1806 (KM452399) from GenBank and matched the BIN for *O. sculpus* [BOLD:ABX3754] from BOLD, further supporting our morphological species identification.

Distribution. Palearctic, including Malta (newly recorded), and introduced into North and South America, New Zealand, Australia, and the Hawaiian Islands (Newton 2019).

Discussion

Seven species of coleopterans are newly recorded from Malta in this study. All species presented here are generally distributed on the European mainland or have a Mediterranean distribution (de Jong et al. 2014), and these species are expected to be present on the Maltese Islands. Our new data represent geographic range extensions for four of these species. Our record of *Cercyon quisquilius* from Malta represents the first from the central Mediterranean, with the closest previously known occurrence being in northern Italy (GBIF Secretariat 2019). Our new data for *Lebia cruxminor* and *Oxytelus sculptus* represent the first records from a Mediterranean island, as both species have been previously recorded only from the Mediterranean mainland (GBIF Secretariat 2021a, 2021b). In addition, our discovery of *Smicronyx pauperculus* from Malta represents its first European records; the closest occurrences are in Israel (Haran et al. 2017).

Each of these newly recorded beetle species was only detected from a single locality on the Maltese Islands (Fig. 1), despite that our sampling efforts were throughout the islands. This observation is likely because these species are locally rare and have a restricted distribution in the islands. *Ancylopus melanocephalus* was collected in UV light traps. *Ancylopus melanocephalus* was recorded at a freshwater habitat, which is a rare habitat throughout Malta, and this habitat specificity possibly contributed to its delayed discovery. The closest geographical records of this species, where it has also been recorded in *Phragmites* marshes, are in southern mainland Italy and Sicily (Focarile 1964). *Lebia cruxminor* is distributed across the vast Palaearctic region (GBIF Secretariat 2021a); however, it believed to be a rare beetle species throughout its native range (Alexander 2003; Dekoninck et al. 2019). We captured *L. cruxminor* in a UV light trap set up in a Mediterranean garrigue habitat. This species is associated with meadow habitats in Ireland (Alexander 2003) and grasslands in Belgium (Dekoninck et al. 2019). Although mainland European grasslands and Mediterranean garrigue habitats are composed of different plant communities, they share an open habitat type and are characteristically sparsely covered by shrubs and herbs.

In conclusion, our discoveries clearly demonstrate that the coleopteran fauna of the Maltese Islands merit further attention, as the lack of knowledge on island faunas is considered a crucial issue for biodiversity conservation (Gillespie and Will 2018). Our study provides new essential knowledge useful for conservation of biodiversity by providing accurate data on species that were previously overlooked. Additionally, our study contributes towards biodiversity conservation by providing DNA barcodes useful for the identification of coleopteran species and for various future biomonitoring applications across Malta, the central Mediterranean region, and beyond.

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Authors' Contributions

Conceptualization: NV, AV. Formal analysis: CMM. Funding acquisition: CMM, NV, AV. Methodology: CMM. Resources: NV, AV. Supervision: AV. Writing – original draft: CMM, AV. Writing – review and editing: CMM, NV, AV.

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